酵素純化與分析

Enzyme Purification and Analysis

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酵素純化與分析

B2

- 酵素純化方法 Enzyme purification methods
- 酵素分析方法 Enzyme analysis methods
- 問題集 Problems

酵素分析方法 Enzyme analysis methods

1. 蛋白質定量法 Protein determination
2. 酵素活性測定法 Enzyme activity assay
3. 電泳檢定法 Electrophoresis
4. 分子量決定法 Molecular weight determination
5. 蛋白質構造與組成分析
   Protein structure and composition analysis
6. 免疫學工具的利用 Immunochemical tools
7. 蛋白質科技 Protein technology

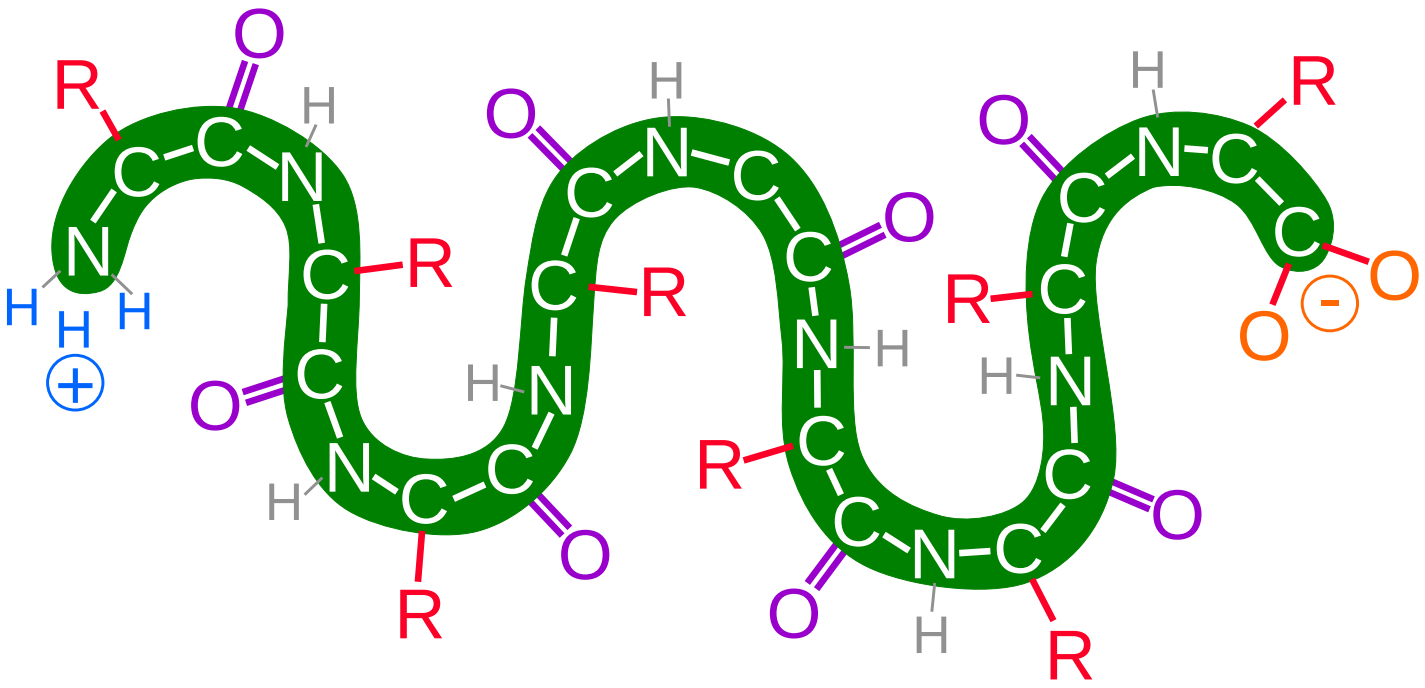
1 蛋白質定量法 Protein determination methods

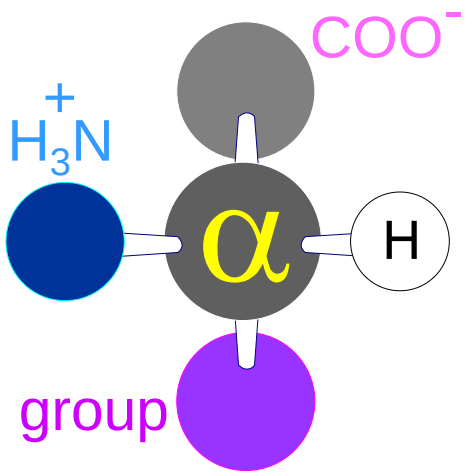
- 1.1 Biuret method
- 1.2 Lowry method
- 1.3 UV absorbance
- 1.4 Coomassie Blue (dye binding) method
- 1.5 Other methods

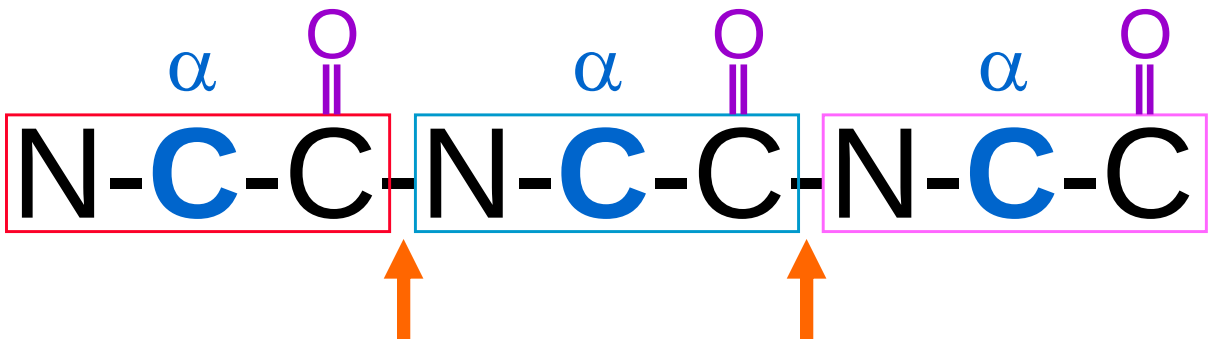
蛋白質構造的骨架 Backbone of protein molecule

Constant

Variable







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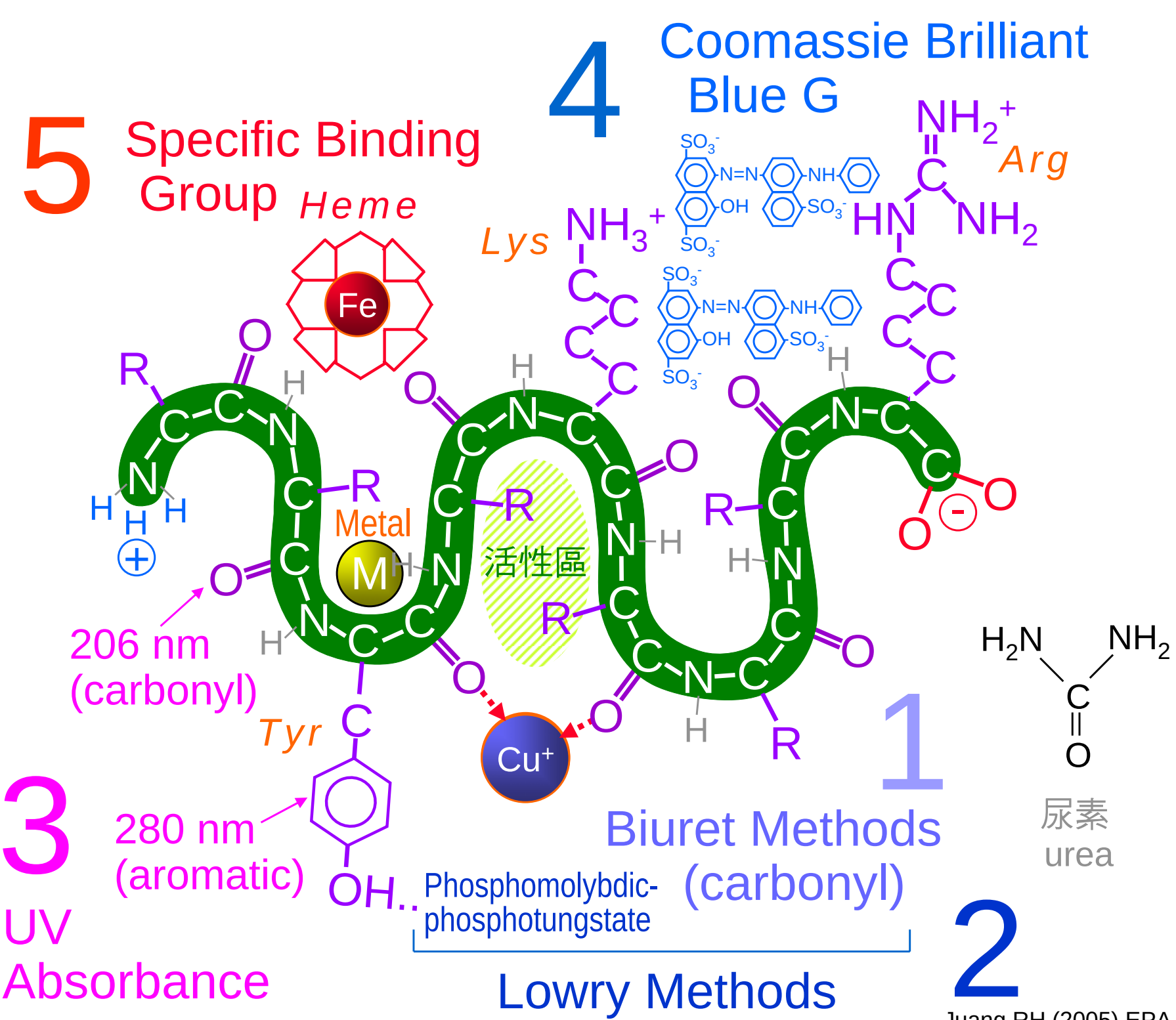
各種蛋白質定量法原理



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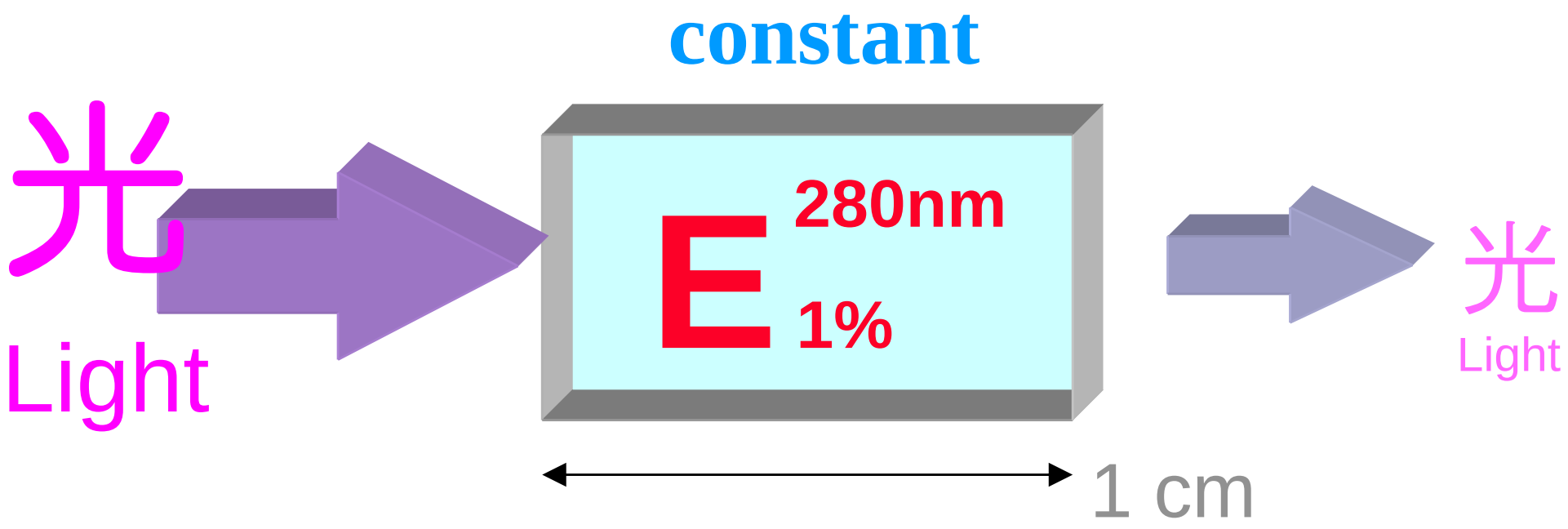
分子消光係數 Molar extinction coefficient

- A constant indicating the capacity of light absorbance for a molecule



吸光值 $A = E \times b \times c$

1 *10* *0.1%*

蛋白質消光係數 UV absorbance by proteins

- 280 nm - Aromatic Groups (Side chain)

  1 mg/mL 溶液→ 吸光度 (280 nm) = **1** 約值

- 192 nm - Carbonyl Groups (Backbone)

  1 mg/mL 溶液→ 吸光度 (192 nm) = **60**

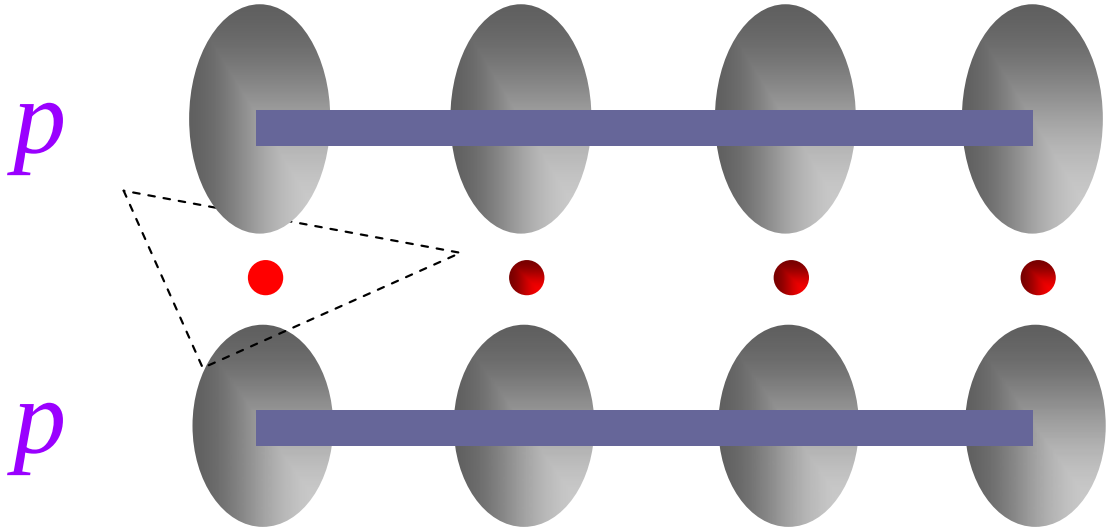
  (206 nm) = **29**

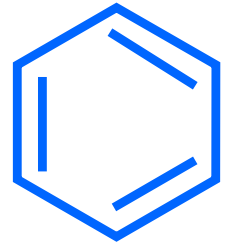
200 nm UV light is interfered heavily by O_2

共軛雙鍵 Conjugated double bonds

sp^2 sp^2 sp^2 sp^2

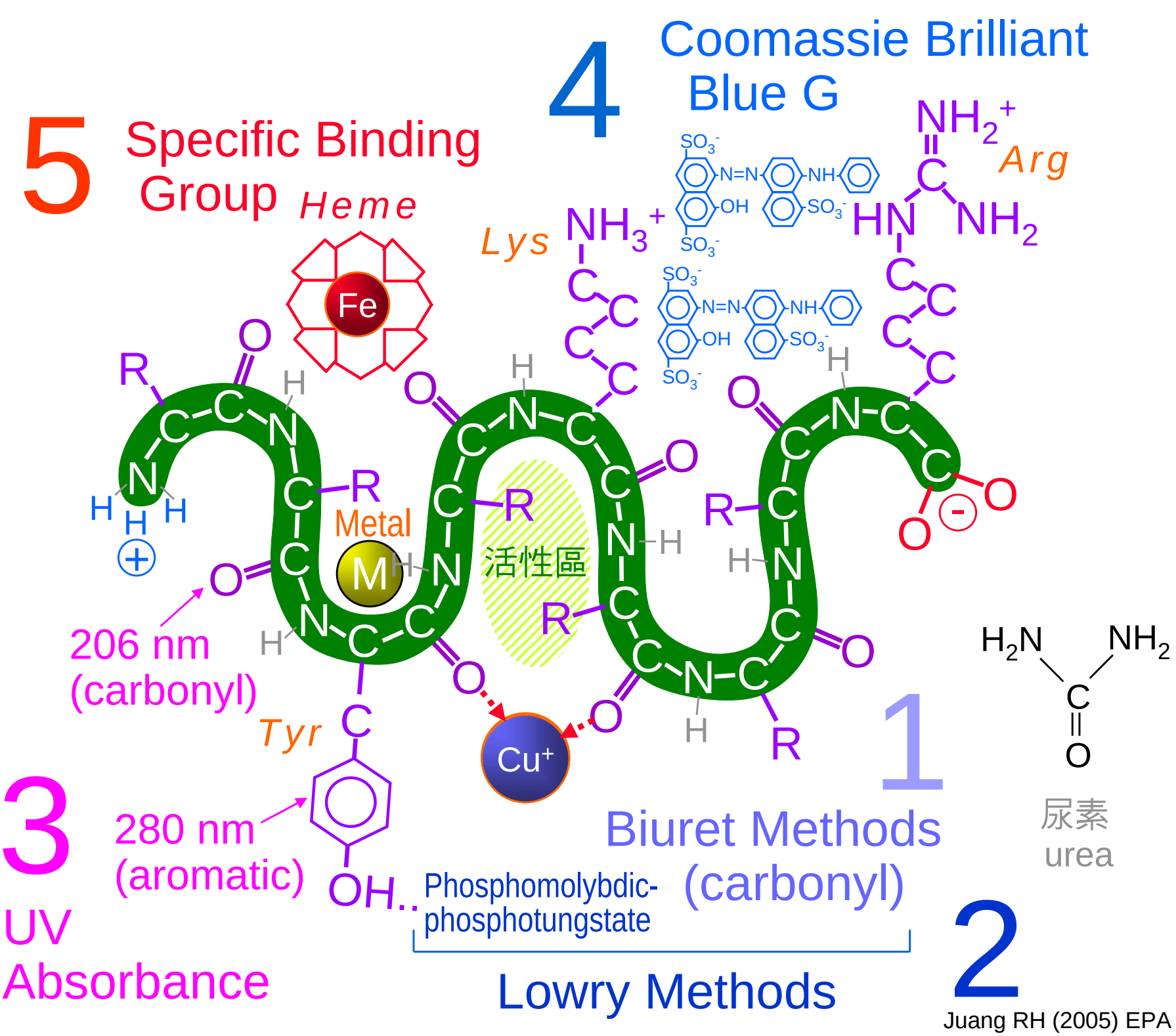
—C=C—C=C—





p 電子共振 resonance

各種蛋白質定量法原理



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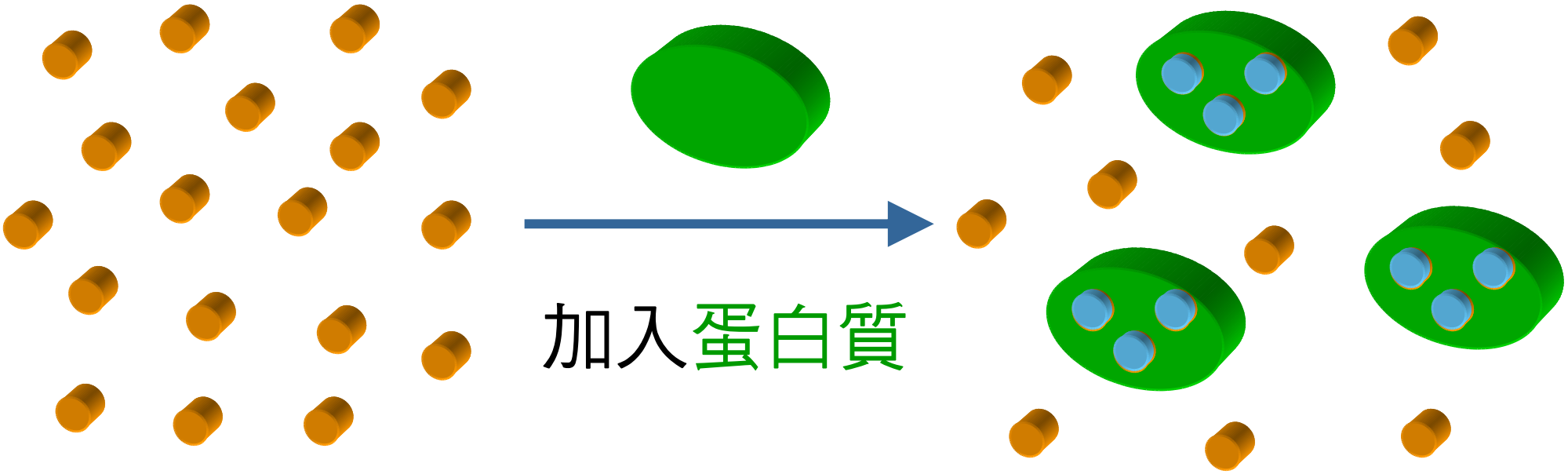
Bradford Method

Coomassie Brilliant Blue G-250

470 nm　　CBG is an *indicator*　　595 nm



酸性環境下呈茶色
Brown (acidic)

與蛋白質結合變藍色
Blue (pH ↑)

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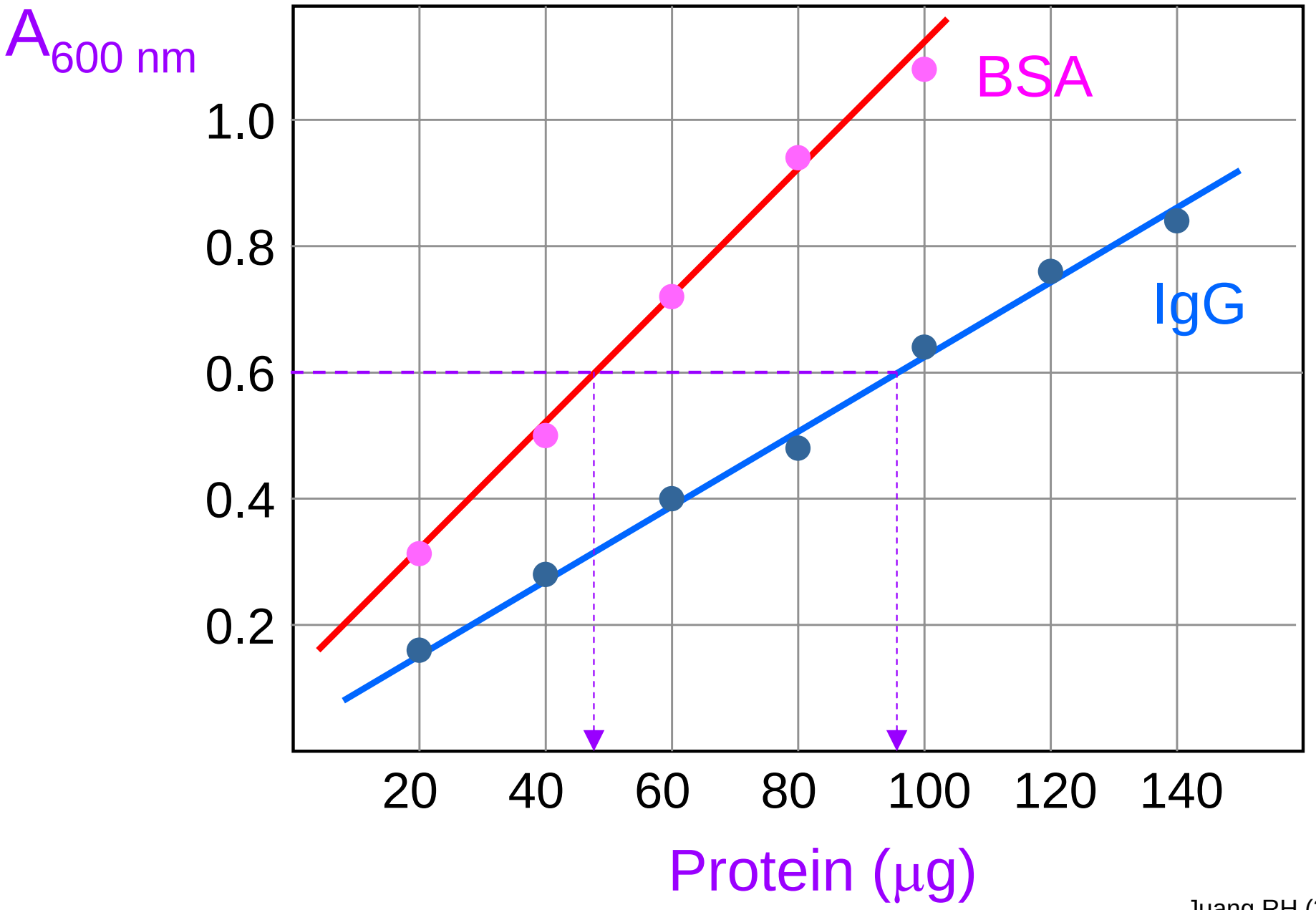
不同蛋白質的定量差異 Deviation of standards



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各種蛋白質定量法的比較

Methods	Precision	Accuracy	Remarks
Biuret	0.05 - 5 mg	High	Rapid, Corrosive, Interference
Lowry	0.05 - 0.5 mg	Medium	Slow, Interference
Absorbance 280 nm	0.05 - 2 mg	Low	Sample recoverable, Interference
Absorbance 205 nm	0.01 - 0.05 mg	High	Sample recoverable, O_2 interference
Bradford Dye-binding	0.01 - 0.05 mg	M - H	Rapid, Interference, Color staining

Comparison of the protein determination methods

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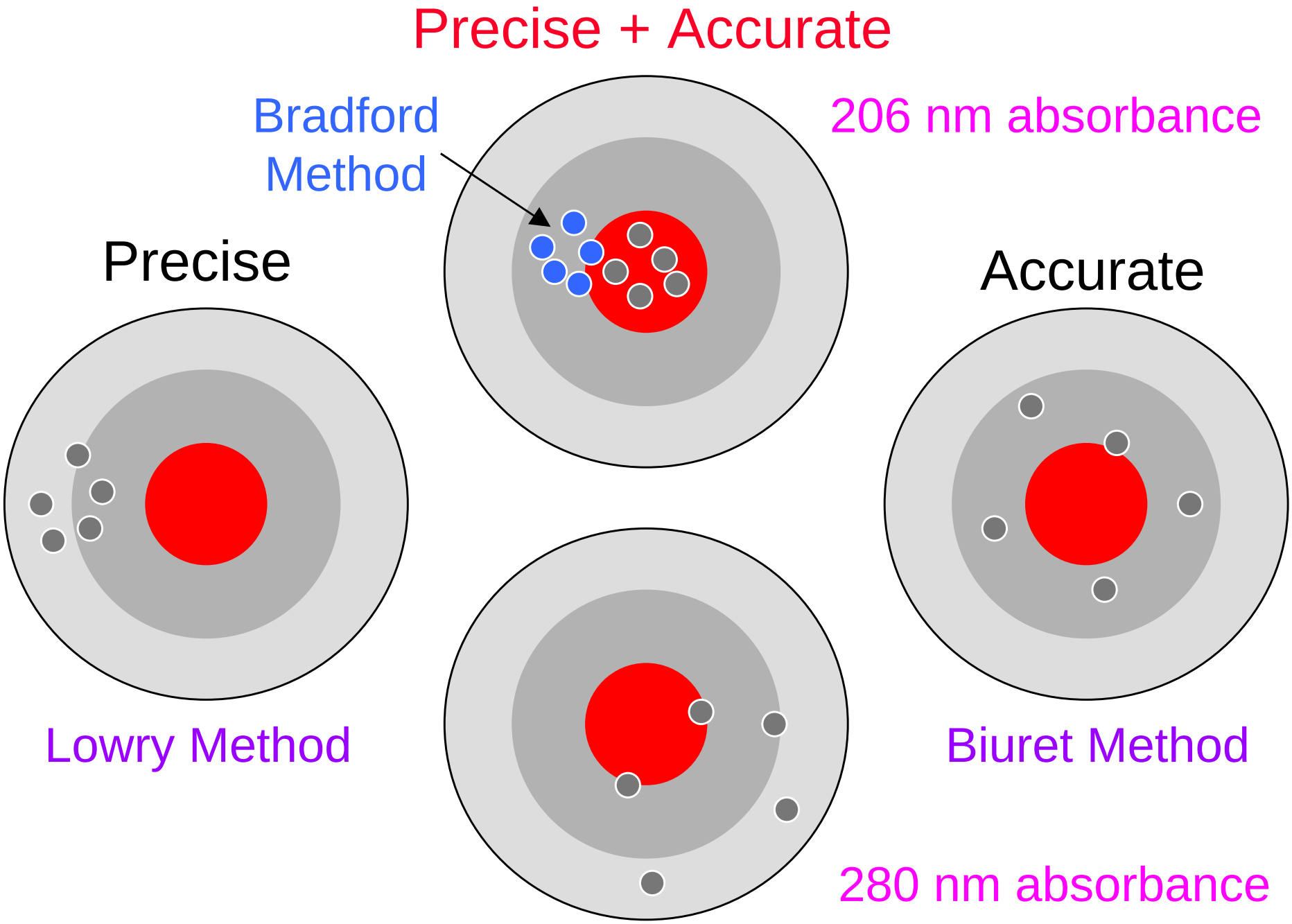

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